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The Concepts of Aposematism and Chemical Defense in Butterflies: Great Misconceptions or Scientific Fantasies?

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Abstract: It is a generally accepted concept that certain secondary plant metabolites, the cyanogenic glycosides, the cardiac glycosides and the pyrrolizidine alkaloids, are the main chemical compounds providing certain Lepidoptera – the Heliconiinae, Acreinae, Ithomiinae, *Danaus plexippus* (LINNAEUS, 1758) and the day-flying Zygaenidae with a potent chemical defense against their avian predators. In this paper the validity of the theory of Aposematism in butterflies is challenged: the concept of chemical defense in butterflies, is considered a misconception; it is argued that insectivorous birds are not capable of tasting the plant metabolites believed to provide butterflies with a potent chemical defense, and thus the concept that beak marks on the wing of a butterfly are a proof of taste rejection of the prey by the avian predator is not valid. It is another misconception. The foraging behavior of an insectivorous bird is discussed in light of the Optimal Foraging Theory (STEPHEN & KREBS, 1986). For a bird to survive, the energy gained during foraging has to be higher than the energy lost. Prey selection depends first and foremost on energetic profitability. Thus a palatable but energetically unprofitable butterfly will be avoided, regardless of its apparently aposematic color pattern. There is a positive correlation between energetic profitability and the morphological and flight characteristics of butterflies. Heliconiinae and Itomiinae are avoided under natural conditions not because they have the protection of a chemical defense and conspicuous considered aposematic color patterns but because they are not energetically profitable to pursue and eat. It is argued that our knowledge of the purported chemical defense of the monarch butterfly [*D. plexippus* (L.)] provided by the cardiac glycosides (CDGs), sequestered from the host plant during the larval stage of development of the butterfly, is based solely on specially designed and controlled laboratory experiments creating conditions that do not exist in the natural environment. A revision of the currently accepted theories and concepts is suggested.

Introduction: Birds are amazingly capable of adapting to even most unnatural experimental conditions to survive. However, under such conditions they can change their normal foraging behavior and act in a way that can be very different to their common behavior in their normal environment, in nature. In this paper I discuss the interrelationship between the avian predators (aerial hawker insectivorous birds) catching their prey on the wing and butterflies as prey in their real natural environment. I will avoid geting involved in the history of the theory of aposematism and that of chemical defense in butterflies but challenge the validity of both these concepts as applied to butterflies. I consider them misconceptions.

My thesis is based on many experiments performed by a great number of scientists, mostly in the 2nd half of the last century, leading to the accumulation of numerous most important data about the metabolic processes in different butterflies, their palatability for birds, the biochemistry of ß-

glycosides and other plant metabolites, the taste ability of birds, avian vision etc. Many of these most important data were neglected or even disregarded by most of the scientists who contributed to today's general understanding of aposematism and chemical defense in butterflies. All this in addition to the dogmatic application of the theory of aposematism to butterflies, led to many misconceptions. In this paper I present arguments that: 1) the very conspicuous color patterns of Heliconiinae and Ithomiinae, and those of the monarch butterfly are not aposematic; the theory of aposematism is not valid for them. 2) Despite the presence in Heliconiinae of theoretically very toxic, specific for the species, ß-glycosides, which are dogmatically considered to provide them with a potent chemical defense, these butterflies are not toxic for their avian predators. Instead they are edible as prey. 3) The avian predator does not possess a specific for the Heliconiinae ß-glycosides enzyme system (glycosidase) necessary for enzymatic hydrolytic release of free cyanide from the glycosides of the prey. Without enzymatic release of free HCN, the ß-glycosides cannot provide the butterfly with a chemical defense. 4) The cardiac glycosides (CDGs) do not provide the monarch with chemical defense. 5) The beak-mark hypothesis, considered a proof of taste rejection of a butterfly, is a misconception. Thus I challenge the validity of the concept that Heliconiini and other neotropical butterflies are aposematic (warningly colored) chemically protected insects.

Are Heliconiini Aposematic Chemically Protected Butterflies?

Heliconiinae (Nymphalidae, Lepidoptera) comprise a large group of Neotropical butterflies with markedly conspicuous, universally considered aposematic, color patterns. They are even given as example of aposematic butterflies (see BROWER, 1963). These butterflies contain specific for the species ß-glycosides (cyanogenic glycosides) (NAHRSTEDT, 1985, 1987; NAHRSTEDT & DAVIS, 1981, 1983, 1985), that are also widely considered to provide these butterflies with a chemical defense against their avian predators. A characteristic for Heliconiinae is that they do not sequester the ß-glycosides from the cyanogenic host plant in the Passifloraceae (passion flowers) but synthesize de novo their specific ß-glycosides. (NAHRSTEDT, 1985, 1987; NAHRSTEDT & DAVIS, 1981, 1983, 1985). The Passifloraceae is unusual in producing cyanogenic glycosides with a cyclopentene moiety (SPENCER, 1988), and hence, are structurally and biogenetically unrelated to the ß-glycosides of Heliconiinae (TANTISEWIE et al., 1969; CONN,1980). The cyanide is derived from the glycosides linamarin and lotaustralin (NAHRSTEDT & DAVIS, 1981). The presence of very low amount of linamarin has been reported for only a few species of Passifloraceae (FUNG et al., 1981; FISCHER et al., 1982). As a result, in Heliconiinae the specific ß-glycosides arise only through de novo biosynthesis (NAHRSTEDT & DAVIS, 1983, 1985). Thus, a characteristic for Heliconiinae is that they do not sequester the ß-glycosides from the cyanogenic host plant in the Passifloraceae but synthesize de novo their specific ß-glycosides.

The fact that Heliconiinae lay their eggs only on specific for the species/race Passifloraceae host plant and have developed enzymatic pathways for de novo biosynthesis of their specific ß-glycosides, instead of sequestering the glycosides from the cyanogenic host plant, indicates that these plants are indispensable for their metabolism and thus for their normal development, but not for providing them with chemical defense. It would be energetically less expensive to use the cyanogenic glycosides of the host plant than for the butterfly to develop its own pathways for synthesizing them de novo. It is energetically costly to biosynthesize these chemical compounds.

The larva of Heliconiinae, the primary herbivores of the genus Passiflora (BENSON et al., 1976), possess a mechanism for deactivation of the plant ß-glycosides of the host-plant that allows them

to use the plant as food. They metabolize the host plant without releasing hydrocyanic acid (HCN), which is the highly toxic component of the glycosides (for details see SPENCER, 1988).

It is logical to assume that before evolving conspicuous coloration, the ancestral form of Heliconiinae was cryptic. Crypticity provides an edible butterfly with the best protection from being attacked by predatory birds. Otherwise, we have to assume that the ancestral form possessed a conspicuous color pattern. It is logical to assume also that the host plant of the ancestral form was in the Passifloraceae, as those of the present Heliconiinae, and that the ancestral form biosynthesized its own specific ß-glycosides also, instead of sequestering that of the host plant. If Heliconiinae are edible butterflies (see CHAI,1986), we have to assume that the ancestral form was also edible, despite the specific glycosides they contained. If the ancestral form was cryptic, what evolutionary forces could lead to replacement of a markedly protective cryptic coloration with one that advertises to predators the existence of an edible prey? It cannot be the result of mimicry. Some Ithomiinae are believed to mimic some tiger-form Heliconiinae. Why should an Ithomiine butterfly mimic a Heliconius? What is it gaining? As I will argue, both subfamilies are falsely considered to be chemically protected from their avian predators, their coloration patterns are not aposematic (warning color patterns), and what is more important, they have similar anatomical features and flight patterns, which, as I argue in the following paragraphs of this paper, determine the foraging behavior of the hunting aerial hawker insectivorous bird. Scientists (not this author), who were nurtured with the theories of aposematism and mimicry, are inclined to see mimicry in every similarity between two species belonging to closely related genera of families of butterflies dogmatically considered to be chemically protected.

If the conspicuous color pattern evolved first, i.e., before the evolvement of toxicity, it cannot be aposematic. To be aposematic, the color pattern has to have a signaling function (warning coloration), i.e., to advertise to predators that the bearer of the conspicuous color pattern is noxious. If the butterfly is edible, i.e., innoxious, despite having a conspicuous color pattern, it will be attacked by bird predators and eaten. Does the postulated aposematic coloration evolve to advertise an illusive toxicity? Obviously no. Conspicuous coloration alone cannot provide Heliconiinae with protection. There are many conspicuously colored palatable butterfly species that are included in the regular diet of insectivorous birds e.g., the bright yellow and black Tiger Swallowtail, *Papilio glaucus* (LINNAEUS, 1758) or *Agrias* butterflies, which are with color patterns far more conspicuous than some *Heliconius erato* (LINNAEUS, 1758) and *H. melpomene* (LINNAEUS, 1758) races. If avian predators avoid all butterflies with conspicuous color patterns, they will lose an important food source. Nature will hardly tolerate this.

Do ß-glycosides Provide Heliconiinae with a Potent Chemical Defense against Avian Predators?

The specific ß-glycosides can provide Heliconiinae with a chemical defense only if the predator possesses a specific enzyme system (specific ß-glycosidases) that releases cyanide from the specific ß-glycosides of the butterfly upon enzymatic hydrolysis. The enzymatic hydrolysis is highly substrate specific (SPENCER, 1988). ß-glycosidases show an exceedingly high degree of specificity toward a particular substrate (HOSEL & CONN, 1982; DALE et al., 1985). I consider the assertion that cyanogenic glycosides release HCN on hydrolysis in the acidic environment of the stomach (BROWER, 1984) a misconception. If this should be the case, a bird that swallows a Heliconius would be a very sick or a dead bird. We eat the delicious passion flowers fruits just because HCN cannot be released in the acidic environment of the stomach.

It has been experimentally proven that Heliconius are not toxic for birds. CHAI (1986), using

neotropical jacamars, one of the most specialized insectivorous bird species that in the field entirely avoids Heliconius, unequivocally demonstrated that in cage experiments, after a long period of food deprivation, jacamars accepted these aposematic, considered very toxic butterflies, as food. One bird in one hour ate 4 *Heliconius*, and during the experiment a total of 9 (nine!) without any sign of sickness. The second experimental bird ate the presented *Heliconius* butterflies after consuming all palatable butterflies presented together with them. Despite this undisputable experimental demonstration that Heliconiinae are perfectly palatable insects, they are still considered to be as one of the best examples of aposematic chemically protected butterflies.

CHAI's experiments unequivocally demonstrate that a bird remains unharmed, without any signs of sickness, even after eating nine *Heliconius*. Thus, if the avian predator does not possess an enzyme system that is capable of releasing HCN from the butterfly's specific ß-glycosides, as I argue is the case, these glycosides cannot provide Heliconiinae with a chemical defense. The glycosides lose their assumed protective function (chemical defense), and the markedly conspicuous coloration loses its warning signaling function also, i.e., it is not aposematic coloration. However, despite that Heliconiinae are not aposematic chemically protected butterflies, the avian predators, under normal natural conditions, still avoid these butterflies. This unexpected behavior of the birds, I argue, can very well be explained on the basis of the principles on which the Optimal Foraging Theory (STEPHEN & KREBS, 1986) is based.

The presence of an enzyme system in birds that is capable of releasing HCN from the butterfly's specific ß-glycosides, has never been tested. The presence of such a specific enzyme system, especially in the buccal cavity and tongue of the bird, has never been experimentally proven. It was never tested! Without experimental proof that birds possess specific ß-glycosidases, the whole concept that Heliconiinae are aposematic butterflies and that the ß-glycosides provides them with a potent chemical defense falls apart.

Beak marks are considered dogmatically as proof of taste rejection of the butterfly by avian predators (POUGH & BROWER, 1977; BOWERS & WIERNASZ, 1979). The beak mark hypothesis is based on the presumption that the bird is capable of tasting a small piece of wing, 2x3 mm in size (the size of an usual beak mark damage on the wing). The beak mark hypothesis to be a valid concept requires: 1) the outer surface of the wing to contain water soluble ß-glycosides molecules, which the bird is able to taste. 2) the avian predator to possess specific glycosidases that liberates free HCN from the ß-glycosides in the small piece of wing held with the tip of the beak. This is where the beak gets in touch with the small beak-mark piece of wing and where the enzyme/ substrate reaction should take place. The reaction must proceed in seconds in order to allow the bird to release the butterfly unharmed. Otherwise, it will devour the prey. However, there is no way for ß-glycosides molecules to reach the outer surface of the wing. Being water impermeable this surface does not contain nor retain water soluble substances under natural conditions. Also, there are no taste buds on the cornified tip of the tongue and no salivary glands, which would create the necessary environment for an enzyme to be functional (see KASSAROV, 1999 and the many references cited). Thus, a prerequisite for the specific ß-glycosides to provide the Heliconiinae (the prey) with a chemical defense is for the bird predator to possess an enzyme system capable of releasing HCN from the ß-glycosides of the butterfly. It does not.

Evidently, even if we assume that the bird possesses an enzyme system capable of releasing free cyanide from the ß-glycosides of the butterfly, the bird is not capable of tasting the ß-glycosides via a beak mark piece of wing. If the bird cannot sense the very toxic cyanide, it will swallow the

prey. Birds swallow the food. They do not chew. A swallowed butterfly is a dead butterfly. Thus, I consider the concept that beak marks are a proof of taste rejection of a butterfly by an avian predator as another misconception that markedly contributed to the misconception that Heliconiinae are chemically protected butterflies.

A beak mark on the wing only shows that the butterfly was caught by the avian predator, but managed to escape, losing only a very small part of the wing held firm by the bird. This is especially valid for Heliconiini. A characteristic for these butterflies is that they have markedly elongated wings (wingspan averaging 6-7 cm for a flying butterfly with open wings). The longer the wing, the more fragile it is at the point where it is held only 2-3 mm inside the beak, and the easier it will break at this point, thus allowing the butterfly to escape practically unharmed. It is not a passive but an active escape; the insect is actively trying to liberate itself from the grip of the beak. It is not waiting for the bird to release it after tasting it. Whether it is a beak mark defect or a beak torn defect (see POUGH & BROWER, 1977; BOWERS & WIERNASZ,1979) the small defect, which remains on the wing after the butterfly escapes, depends on only, I argue, whether a vain of the wing was held in the grip of the beak or not. I do not agree with these authors and BROWER (1984) that beak mark butterflies are actively taste-rejected by the bird, whereas beak torn butterflies were caught and would have been eaten, but escaped by breaking away from the beak of the bird. This misconception is based on the firm belief that birds are capable of tasting a butterfly whether it is chemically protected or not.

According to the Optimal Foraging Theory (STEPHEN & KREBS, 1986), a prey can be perfectly edible for a bird, but the bird may choose to avoid it if it is not energetically profitable as food. A bird will not spend precious energy to pursue a prey if the energy gain is less than the energy spent (negative energetic balance). Thus, efficient foraging necessitates: 1) that the bird be able to recognize profitable prey (energetic profitability) and attack quickly before it escapes, and 2) that the bird does not lose energy to pursue and hunt unprofitable prey. For the bird to survive, the energy gain during foraging has to be higher than the energy loss. Prey selection depends first and foremost on energetic profitability (ZACH & FALLS, 1978). Foraging predators switch between multiple prey types according to their relative benefits and cost, or profitability, i.e., caloric gain per unit handling time. The relative profitability of morphs determines which morph should be attacked when detected. Of two prey types, the currently less profitable prey should start to be attacked when the encounter (and detection) rate of the more profitable prey is less than a threshold (ZACH & FALLS, 1978; STEPHEN & KREBS, 1986; see the review of the Optimal Foraging Theory by PYKE et al., 1977). Accordingly, an experienced bird will not spend energy in pursuing an insect with a marginal profitability as energy source, except if it is hungry or if there is no other choice of food as, for example, under controlled experimental conditions. The validity of the theory postulated by STEPHEN & KREBS, (1986) was unequivocally experimentally proven by CHAI by using hungry birds as predators of butterflies. Obviously, Heliconiinae are not chemically protected butterflies, i.e., the ß-glycosides do not provide them with any chemical defense.

In general, butterflies thought to be unpalatable in the literature are characterized by a long narrow abdomen, narrow thorax, elongated wings and fluttering wing beats, and a characteristic slow flight in a straight and regular path, which is easily recognizable by the bird predator. They are easy to catch. In contrast, considered palatable butterflies have a shorter and stout abdomen, wide thorax, relatively shorter wings, and a fast, evasive irregular flight, making them difficult to catch; they more easily escape when attacked (MARSHAL, 1909, CHAI, 1986, 1988,1996; CHAI & SRYGLEY, 1990;

PINHEIRO, 1996). Most of the wide thorax cavity of the palatable butterfly is filled with massive flight muscles (protein, high nutritional value) for quick take off, acceleration and increased flight speed (CHAI & SRYGLEY, 1990). In contrast, the thinner thorax of the hypothetically unpalatable butterflies is associated with their slow, more regular non-evasive flight, and contains weak flight muscles (less protein, low nutritional value). The longer and more slender the abdomen, the more the indigestible chitinous cuticle mass in relation to the digestible abdominal content, i.e., the less the nutritional value per body mass, the less profitable as food. The amount of digestible tissue in the shorter and stouter plump abdomen of the palatable butterflies is significantly higher in relation to the cuticle: accordingly, the higher the nutritional value of the butterfly as food. Evidently, a bird quickly learns to associate the morphological characteristic of the prey and its characteristics flight pattern with its nutritional value (energetic profitability as food), with palatability (see CHAI, 1990; CHAI & SRYGLEY, 1990). It becomes an experienced predator.

The positive correlations between palatability and unpalatability of butterflies and their morphological and flight characteristics described by CHAI & SRYGLEY (1990) are in full concordance with the Optimal Foraging Theory on which my thesis is based. I do not agree with the terms palatability/ unpalatability that these authors used. In fact, what CHAI & SRYGLEY observed is a positive correlation between profitability (energetic profitability) and the morphological and flight characteristic of butterflies. Thus a palatable but energetically unprofitable butterfly will be avoided, regardless of its incorrectly viewed aposematic color pattern. Heliconiinae (also Ithomiinae) are avoided under natural conditions, not because they have the protection of a chemical defense and conspicuous (aposematic) color patterns, but because they are not energetically profitable to eat. Thus, a butterfly cannot gain protection against avian predators by mimicking the color patterns of Heliconius butterflies. To gain protection, it should mimic their morphological and flight patterns, i.e., it should mimic their energetic unprofitability. HENRI BATES (1862), who was puzzled why *Dismorphia* (Pieridae) look and behave like Heliconiinae and Ithomiinae, wrote his famous paper on protective mimicry now called Batesian mimicry. He concluded that the *Dismorphia* butterflies mimic the warningly colored and unpalatable ithomiine and Heliconius butterflies to gain protection from predators. I present a different point of view: Dismorphinae are avoided by avian predators not because these butterflies, in order to get protection, mimic the bright conspicuous color patterns of the wrongly considered unpalatable Heliconiinae and Ithomiinae, but because, as the Heliconiinae and Ithomiinae, they are energetically unprofitable to eat. Many species of the genus *Dismorphia* (Pieridae), which are widely considered to mimic ithomiine butterflies (mainly Mechanitis and Hypothiris) have a slender elongated body, tiny narrow thorax, very small head, and a slow regular flight pattern.

In fact, the first thing what a hunting insectivorous bird catching its prey on the wing sees from the perch, is not the color patterns of an approaching flying butterfly, i. e., whether it is aposematic or not, but its characteristic flying patterns. From a distance, I argue, the bird does not see the distinct color patterns of the wing, but sees an additive mixture of colors (see KASSAROV, 2004). In that paper I discussed in detail the theory of aposematism, mainly from the standpoint of the visual abilities of the avian eye, how the bird sees the color patterns of a flying butterfly. To increase the chance to catch the prey before it disappears in the vegetation, especially in the dense vegetation of the jungle, the bird must be capable in seconds of taking a decision to attack or neglect a potential prey. From its flying pattern, not from its coloration patterns, it recognizes whether it is a prey energetically profitable or not, whether to attack or neglect it. Butterfly flight patterns function as a

most important visual signal for the bird.

Hunger, however, can change the normal behavior of the predatory bird as shown by CHAI (1986). No other choice of food, or restricted space in the cage making it impossible for the bird to observe from the perch the characteristic flight pattern for *Heliconius* or any butterflies, dead butterflies presented to experimental birds, or butterflies with painted wings, etc., which are conditions typically involved in cage experiments, can force the bird to change its natural habits and eat or avoid prey without reference to normal feeding habits in nature.

The evolvement of conspicuous color patterns and of specific ß-glycosides (hypothetical chemical defense) in Heliconiinae (the prey), and also the evolvement in the predator of a corresponding specific enzyme system for the activation of the potential chemical defense of the prey are events that would need to be synchronized in a way to allow the whole system to be functional. Also, to assume that both pathways, that of the biosynthesis of the ß-glycoside in the butterfly and of the specific glycosidase in the buccal cavity of the bird, evolved simultaneously, is most speculative. It necessitates the participation of many multiple enzyme systems in the prey and the predator that would evolve simultaneously and function synchronously. This has not been shown to be the case and is, indeed, not only purely hypothetical but antithetical to the experimental evidence by CHAI, (1986) and CHAI & SRYGLEY (1990) and the anatomical and physiological evidence from birds in general.

Two questions arise: what evolutionary forces could lead the avian predator to evolve an enzyme system that deprives it of food by activating an otherwise inactive chemical defense in the prey? There is no need for the bird to develop an enzyme system that is able to release HCN from the ß-glycosides of the prey. What is the reason for Heliconiinae to develop aposematic color patterns if their main predators (ants and parasitoid predators) and less important vertebrate predators (insectivorous birds) are not able to taste their hypothetical toxicity?

Do Pyrrolizidine Alkaloids (PAs) Provide Ithomiinae with Chemical Defense?

Ithomiinae (Nymphalidae) is another subfamily of about 400 species of Neotropical butterflies comprising many genera with conspicuous color patterns that are generally considered to be aposematic and chemically protected against avian predators. Pyrrolizidine alkaloids (PAs) are the factor supposed to provide these butterflies with a potent chemical defense. The Solanaceae host plants of Ithomiinae do not contain PAs and, in contrast to Heliconiinae, which biosynthesize their specific ß-glycosidases, Ithomiinae do not biosynthesize PAs. They acquire PAs from foreign sources: flower nectar (Compositae: Eupatoriaceae) or decomposing foliage (Boraginaceae) that they avidly visit soon after emerging from the pupa (BOPPRÉ, 1984; BROWN, 1985, 1987). The imago, newly emerged from the chrysalis, does not contain PAs in the body and wings. Obviously, the Ithomiinae species, which possess conspicuous, considered warning coloration patterns, despite these color patterns, remain unprotected by a hypothetical chemical defense. They are perfectly edible in the most vulnerable period of their life during and after emerging from the chrysalis and their first adult flight to the external source of PAs which they have first to locate. Evidently, if PAs are the chemical substances providing Ithomiines with chemical defense, at this time of their life, the conspicuous coloration patterns are not aposematic. Again, to be aposematic the coloration patterns have to have a signaling function: to advertise to predators that a potencial prey is noxious (in the case of diurnal butterflies – the presence of a chemical defence). What are the color patterns of conspicuous Ithomiine butterflies at this critical period of their life advertizing?

Their vulnerability? Amazingly, there are many genera of Ithomiinae with transparent wings that almost perfectly blend into the surrounding habitat. Their crypticity actually provides them with better protection against avian predators than the conspicuous color patterns of the hypothetically aposematic genera.

PAs serve a most important biological function of Ithomiinae. The ♂♂ of this subfamily of butterflies use PAs for pheromone synthesis (see BROWN, 1987). ♂♂ are the only sex found on foreign sources of these alkaloids (PLISKE, 1975a, b). The ♀♀ of most species seem to acquire PAs from the spermatophores received from ♂♂ during mating. These small sacs often have 20-50 times the PAs concentration as the remainder of the ♂ body (BROWN, 1984, 1985).

Among vertebrate animals, PAs are converted to pyrrols, which produce hepatogenic, mutagenic, oncogenic and other deleterious effects (BULL et al., 1968; MATTOCKS, 1968). PAs are very slow acting toxins and, in contrast to cardiac glycosides (CDGs), they have no emetic properties and do not manifest symptoms of sickness, not even malaise, for months after ingestion. Therefore, PAs are unlikely to provide any chemical defense of butterflies against vertebrate predators other than a deterrent effect derived from their very bitter taste. To serve as a chemical defense against avian predators, birds should be capable of tasting the bitter taste of PAs, and taste-reject the prey items without disrupting the integrity of the integument. Thus, the only way avian predators can taste-reject an Ithomiine butterfly is to be capable of tasting the bitter taste of the alkaloids on the beak-marked piece of wing.

There is no known mechanism of circulation of hemolymph in the distal three-fourths of the adult wing. Wing circulation of hemolymph in the mature imago occurs only in less than 1 cm out from the body. Thus, no PAs from the reproductive system of the ♀ (where the spermatophores obtained during mating deposit PAs) can reach the periphery of the wings of a mature adult. This is the site where the beak-marked piece on the wing is usually located. Evidently, there are no PAs in this part of the wing to be tasted. Besides that, there are no taste buds and salivary glands in the cornified tip of the tongue of the bird, which is the only avian mouthpart to be in contact with the wing of the captured butterfly (see KASSAROV, 1999). How PAs taken from foreign sources reach from the digestive tract the periphery of the wing after hardening of the wing is another puzzle. After hardening of the wing, there is functioning circulating hemolymph only close to the body, mainly to feed the sense organs in the wing base (in all butterflies), and (in some butterflies) the scent-producing glands (SCOTT, 1986).

Thus, most of the arguments that I presented to oppose the validity of the theory of aposematism for Heliconiini and that they are not chemically protected butterflies are true for Ithomiinae also, and as it will be shown, for the monarch *Danaus plexippus* (LINNAEUS, 1758). Not valid for Ithomiinae is also the dogmatically held belief that the beak-mark hypothesis is a proof of taste rejection of the prey by a predatory bird. As with Heliconiinae, under normal natural conditions, Ithomiine butterflies are avoided by avian predators, I argue, not because PAs provides them with chemical protection, or because the bitter taste of these alkaloids, and not because their toxicity is being advertised to predatory birds by aposematic color patterns, but because these butterflies are energetically not profitable for the predator in the same way as in the Heliconiinae. The Optimal Foraging Theory finds another confirmations in Ithomiinae. These butterflies with their small heads, markedly smaller than that of the Heliconiinae, and their significantly thinner thoraxes and abdomens than that of Heliconiinae, renders them even less energetically profitable for avian predators than Heliconiinae.

Is the Monarch *Danaus plexippus* (L.) a Chemically Protected Butterfly? Do Cardiac Glycosides (CDGs) Provide the Monarch with Chemical Defense?

The monarch butterfly is another butterfly considered aposematic and protected by a potent chemical defense. Both sexes of the adult monarch acquire PAs from decomposing plants or nectar of various species of the Asteraceae, Boraginaceae, Apocinaceae and Fabaceae (EDGAR ,1975; EDGAR et al., 1986a, b; PLISKE, 1975; ROTHSCHILD & MARCH, 1978; BROWER et al., 1984; BROWN, 1987). These authors suggested that, once ingested, PAs probably function as part of the butterfly defense mechanism. This suggestion was supported by the fact that the monarch butterfly, unlike many Danaines, is not dependent on PAs as an obligatory precursor of its pheromones (EDGAR et al. 1973; ROTHSCHILD & EDGAR, 1978). EDGAR et al., (1979) speculated that the PAs externally acquired by *D. plexippus* (L.) may be of greater importance for the chemical defense of these butterflies against their bird predators because their larval host plant, *Asclepias* species, offer only an unreliable supply of CDGs (cardiac glycosides). According to BOPPRÉ (1984, 1986, 1990), PAs provide the principal chemical defense of Danainae while only species of the *D. plexippus* (L.) possess the additional defense mechanism based on storage of cardiac glycosides (ACKERY & VANE-WRIGHT, 1984, 1985). ROTHSCHILD & MARCH, (1978) consider PAs also the principal predator deterrent. It has also been suggested that an alternative dual PAs/CDGs-based defense may exist in danaine butterflies (ROTHSCHILD et al.1975; ROTHSCHILD & EDGAR, 1978; BOPPRÉ, 1986)

ROTHSCHILD et al. (1984) found highly odorous methoxy-alkyl pyrazines in monarchs and in moths of the genera Zygaena and Amata, which are sequestered from the host plant (GUILFORD et al., 1987; MOORE et al., 1990). Pyrazines are believed to provide warning signals when paired with a component of a complex chemical defense as that supposed in the monarch.

However, the best evidence that birds are unable to taste the bitter PAs, or that the bitter taste does not deter them from attacking and eating butterflies at all., is from *D. plexippus* (L.) itself. Despite the presumed aposematic coloration pattern, the very bitter taste of both PAs and CDGs, and the odor of pyrazines, the monarch remains a palatable butterfly until the bird ingest enough CDGs to experience the effect of vomiting, which is a symptom of toxicity of these glycosides. Nontoxic monarchs, those that do not contain CDGs (larva feeding on *Passiflora* plant that does not contain CDGs) but still contain PAs and pyrazines, and their presumed aposematic color pattern, are treated by the birds as palatable butterflies and are eaten. This non-toxic class of monarchs are not distasteful to avian predators. I therefore discount the role of PAs in the chemical defense of monarchs at all. I consider the presented indisputable facts as a proof of my thesis that birds are not capable of tasting PAs and other secondary plant metabolites, regardless of their bitter taste, and that the conspicuous coloration patterns of butterflies are not aposematic, i.e., they are not meant to advertise a hypothetical chemical defense in butterflies against their avian predators.

The larva of *D. plexippus* (L.) feeds on milkweed plants (Asclepiadaceae) that contain cardiac glycosides (CDGs), known as cardenolides (PARSON, 1965; EUW et al., 1967; REICHSTE et al., 1968. Monarch larvae ingest and store the CDGs of the host plant. During metamorphosis the sequestered chemicals become generally distributed throughout the tissue and organs of the imago, with the highest concentrations in the chitinous exoskeleton and wings (BROWER & GLAZIER, 1975; BROWER at al.,1984).

Monarchs reared in their natural environments on two *Asclepias* species in California showed a range of 0.28 - 26.0 emetic units (ED50) per butterfly (BROWER et al., 1968; BROWER et al., 1982; BROWER et al.; 1984). Other studies of monarchs collected in the wild from various migrating po-

pulations indicated that the butterflies contain from 0 to over 9 emetic units (BROWER & MOFFIT 1974; FINK & BROWER, 1981). BROWER et al., (1982) found that in Massachusetts about 60% of the wild monarchs contained less than one emetic unit (including some without any CDGs) with the remaining 40% containing more than one emetic unit. How, under these circumstances, the exact amount of CDGs (ED50 units) in a monarch present to the jays in an cage experiment can be established remains for me unexplainable.

Chemical and pharmacological analyses of both plants and butterflies have proven that emesis is caused by the sequestered highly toxic CDGs (BROWER et al. 1968, 1982, 1988). These observations led to the supposition that the presence of cardiac glycosides in the monarch provides it with a highly effective defense mechanism against vertebrate

Blue jays *Cyanocitta cristata bromia* (OBERHOISER, 1921) used as experimental predators, exhibit a typical syndrome of CDGs poisoning, one of the symptoms of which is vomiting (BROWER et al., 1967; BROWER et al., 1968; BROWER & FINK, 1985), but, as I will argue, not in their natural environment, but only in well orchestrated laboratory cage experiments and after a very elaborated special training, or forced feeding of the birds. I consider very important to draw the attention of the reader of this paper that the temperate blue jays, are not insectivorous birds. They are omnivorous, opportunistic feeders (KAMIL & YOERG, 1982). They belong to family Corvidae. Their main food includes different plant and animal sources such as seeds, grains, nuts, wild berries and fruits, small vertebrates, and invertebrates, mainly terrestrial non-insect arthropods and insects. Butterflies are not part of their usual foraging diet, and under natural conditions, jays normally do not prey on monarchs. The aerial hawker insectivorous birds, by contrast, exemplify avian predators specialized in hunting insects on the wing. A very important fact is that these birds, in contrast to blue jays, do not recognize nonmoving or dead insects as prey; they eat only flying insects (see DAVIS, 1977; CHAI, 1986, 1988; CHAI & SRSGLEY, 1990; PINHEIRO, 1996). Prey cannot be passive victims in a predator-prey system, as is commonly implicated by laboratory studies with dead prey as that performed by BROWER at al. 1968, 1982, 1988; BROWER & FINK, 1985). I argue in this paper that in the aerial hawker insectivorous bird/prey relationship the prey plays a very active role in determining whether the avian predator will attack or not attack a potential prey, and whether the encounter will be successful.

It is not incidental that the caged wild-captured jays used in the studies of BROWER & FINK (1985) required a screening period in which each bird, after food deprivation to assure a high level of hunger, was presented with a sequence of dead palatable *Anartia amalthea* (LINNAEUS, 1758) for as many presentations as necessary until the hungry bird consistently accepted and ate these butterflies without hesitation. Obviously, butterflies are not an usual food soarce for the wild jays in their natural environment and they need to be trained to eat them. Then, after food deprivation of 16 hours, each bird was offered its first monarch up to 10 times. If the bird did not attack and eat the monarch during one of these presentations, it was eliminated from the experiment! If it did eat the monarch, the same bird was presented with up to 10 passes of the feeding cup with the second monarch (of the same palatability category as the first) over a one-hour period. If the bird refused to attack this second monarch, it was reoffered again to the bird from passes 11-20. If it again refused to attack, this regimen continued on day 2 for blocks of 10 presentations from passes 21-50. This regimen was repeated for a maximum of 200 passages over 41 days. Without any doubt, results and conclusions based on experiments using hungry, very specially trained jays as experimental birds and dead monarchs as pray, cannot and should not be extrapolated to the foraging behavior of aerial hawker birds in their natural habitat.

They are valid only for the specially trained jays used in the specially designed experiments.

Because of the delayed manifestation of the CDGs toxicity, a toxic monarch, regardless of its degree of toxicity and marked bitterness of the CDGs, remains a palatable food for an inexperienced bird until it experiences the consequences of consuming more than one emetic unit of CDGs, the vomiting. In the controlled cage experiment the only food the hungry, trained to eat dead monarchs, jay faces are toxic monarchs only, not a proportional mixture of different food items similar to those it hunts in its natural habitat. It has no other choice but to eat the presented to him dead monarchs until it vomits. Under natural conditions however, a wild jay does not have in his diet flying monarchs in his diet. Evidently, there is no way for a wild jay to develop conditioned based aversion to monarchs under natural conditions! Thus, it is the used experimental design only that assures the development of condition based avoidance learning in the specially trained hungry jays!

The only way an aerial hawker insectivorous bird could associate the monarch with the vomiting would be to eat several toxic monarchs in quick succession and vomit before it attacks and eats other food. However, because of the delayed manifestation of CDGs toxicity this is hardly possible. The bird has sufficient time to continue foraging and eat a variety of different insects. The monarch is thus an unlikely last meal that the bird could correctly associate with toxicity. Furthermore, in the habitat of an aerial hawker insectivorous bird, and also wild blue jays, monarchs represent only a small proportion of the potential prey population. There is a great variety of alternatives, easier to pursue successfully, catch and subdue, and, what is most important, provide more profitable as a source of energy food that the bird can consume without the long manipulation time (lasting 9.4 - 14.1 min. for a n experimental jay) and during which time it does not hunt, i.e., it is deprived of food. Under natural conditions such a way of foraging should be the most inefficient one, and in full discordance with the Optimal Foraging Theory. Evidently, aerial hawker insectivorous birds in their natural habitat could not develop condition based avoidance learning to monarchs containing SDGs.

Brower & Fink (1985) claim that they have established that food-deprived jays develop conditioned visual aversion to monarchs after consuming a monarch sufficiently toxic to cause vomiting. "For the jays, then the monarchs have distinguishable gustatory properties, presumably as a result of the presence or absence of cardenolides." The question is not whether non-toxic and toxic monarchs have distinguishable gustatory properties, as they certainly must, but whether these authors experimentally proved that under natural conditions the birds can develop conditioned taste discrimination? Development of conditioned taste discrimination, I argue, is possible only under the strictly controlled experimental conditions and the use of blue jays that before the experiment underwent either a most bizarre training period or were forced fed.

Since there is no visual difference between monarchs containing or not containing CDGs, and because the bird swallows the body whole, it is unable to control the amount of toxin entering the gizzard. The vomiting of the experimental jays is not only a manifestation of toxicity, but it is also a vital defensive reaction that prevents the bird from absorbing a lethal dose of toxin, but only in the cage experiment, not in their natural environment. Again, the only way the cardiac glycosides can provide a butterfly with a chemical defense is the naïve bird, not the unique jays used in the cage experiments, to associate the monarch with vomiting, i.e., the consequence of consuming it, and develop constant conditioned visual aversion to all monarchs regardless of their degree of toxicity, i.e., whether they are noxious or innoxious (palatable). The key question remains: is the bird, a jay or aerial hawker insectivorous bird, able at all to develop delayed visual aversion to monarchs in their usual natural

environment as BROWER & FINK (1985) claim to have experimentally proven, but I strongly oppose.

Because the bird is not able to determine on the basis of taste the amount of CDGs a monarch contains, it is most difficult to understand how the Emetic Based Palatability hypothesis postulated by BROWER (1967, 1968) as a theoretical possibility of a palatability spectrum in nature, can operate under natural conditions. During the experiment jays were force fed with gelatin capsules loaded with the desired dry weight of powdered butterfly material and forced down into the gizzard via a tube, a distance of about 12 cm. BROWER considered this hypothesis "a most important ecological discovery: individual insects with 1.0 ED50 CDGs or greater are completely unsuitable as food, where individuals with an ED50 of less than 1.0 could serve as an emergency food supply, provided that a bird ate them at a low enough rate." This hypothesis cannot be valid even for the birds used in the conditioned cage experiments. If a bird develops delayed visual aversion as the result of vomiting, it will lose all monarchs as a food source regardless of the degree of toxicity including the nontoxic monarchs (ED50=0), and thus, they cannot serve as an emergency food supply. Also, how a bird can determine the rate of consumption of monarchs remains another puzzle. In BROWER's hypothesis this is an obligatory condition. The existence of emergency food supplies is implausible under natural conditions. It is another most important misconception, not a most important ecological discovery.

In the experiment of BROWER & FINK (1985) each of the birds was presented with 5 toxic and 15 non-toxic monarchs. The 16 birds killed almost all 80 toxic and 240 non-toxic monarchs and ate 79% of the non-toxic and 9% of the very toxic individuals. Thus, all toxic monarchs containing even >3.0 emetic units of the bitter and highly toxic CDGs were killed and 11 % were eaten. The rest were protected from being eaten, but not from being killed. This would be a most strange chemical defense for a prey species to adopt. Whether the predator does or does not eat the prey after killing it, the prey is a dead prey, i.e., not a chemically protected butterfly.

Under the conditions in their natural environment, birds, especially the omnivorous jays, will never (!) face a situation that leads to severe food deprivation, and then have at their disposal as food only very toxic monarchs (ED50>3.0) and non-toxic monarchs (ED50 = 0.0) without any other source of food, as under the experimental design used by BROWER & FINK. Under such artificial conditions, and after being conditioned to attack both palatable and emetic monarchs, the hungry jays will treat monarchs as potential food, attack and kill them, as the experiment demonstrates. If, however, after the initial vomiting episode, the birds were offered a variety of food items, including monarchs, it is reasonable to assume that they would behave in a very different way. Because of the developed conditioned visual aversion (but under the experimental condition only!), they would avoid the monarchs, whether toxic or not toxic, and eat the other food items.

In the experiment, the ratio of highly toxic monarchs (ED50 > 3.0) to monarchs that did not contain CDGs (ED50= 0.0) was 1:3 (obviously chosen in favor of the nontoxic monarchs). The very hungry birds, trained to eat monarchs, attacked and killed all offered monarchs (non-toxic and toxic). Why did the birds eat 11% of the highly toxic monarchs, while they still had 156 non-toxic monarchs at their disposal, remains unanswered. The chance a toxic monarch being consumed by the birds was significantly lower; the nontoxic monarchs outnumbered three times that of the toxic. What would be the result, however, and the conclusion, if the ratio chosen was 3:1 in favor of the highly toxic instead of the non-toxic monarchs? The birds, forced by hunger, would kill and eat the monarchs. However, because the majority of them were highly toxic, the hungry bird should soon approach a state of steady vomiting. The steady vomiting and long recuperation time would

lead to a marked increase of the degree of hunger, and if the experiment has been continued for a sufficiently long time, the birds should starve to death.

If birds are able, as claimed by BROWER & FINK to reject monarchs at the level of visual unconditioned aversion, why should they bypass this ability and undergo conditioning by getting violently ill (vomiting many times) to reject monarchs? Both conditioned and non-conditioned nondestructive taste sampling require the ability of the bird to taste the monarch. The experiment shows that, during the manipulation time (9.4-14.1 min.), a jay cannot differentiate between a toxic and non-toxic monarch. Evidently, the considered aposematic coloration does not serve as a warning mechanism and the jay cannot taste the very bitter CDGs. If the birds could taste the bitter CDGs (and PAs also), they would not consume monarchs to the point where they finally experience the consequences of their toxicity.

In their experiment to test whether the jays are capable of tasting concentration levels of CDGs below those that lead to emesis, BROWER & FINK (1985) used artificial food consisting of pieces of bread on which solutions of different concentrations of chemically pure pharmaceutical CDGs were pipetted. These glycosides do not belong to the CDGs of monarchs. There are no monarchs with chemically pure CDGs on the outer surface of their body and wings. The glycosides are very tightly bound to the cuticle which is not water permeable. This approach leads to very misleading conclusions as, for example, "Thus the taste rejection threshold for cardenolides in the blue jays is sufficient to allow them to avoid ingesting an emetic dose without prior emetic conditioning."

Thus, our knowledge of the purported chemical defense of the monarch provided by the toxic CDGs sequestered from the host plant during the larval stage of development of the butterfly is based solely on results obtained with specially trained or forced-fed jays and highly controlled laboratory experiments creating conditions that do not exist in the natural environment. The toxic CDGs can provide the monarch, as I claim, with a chemical defense only through delayed poison-based avoidance learning. This mechanism, however, I argue, cannot be operational under natural conditions. I consider the concepts postulated by BROWER & FINK (1985) experimental artifacts. The well known illustrations of a vomiting blue jay do not presents the reality; they are simply experimental artifacts. Under the unrealistic experimental conditions, hunger becomes the dominant factor that suppresses the acquired visual conditioned aversion and dominates the foraging behavior of the hungry bird. The results and conclusions made from such totally unrealistic experimental conditions, creating a fantasy world and also fantasy birds, can be valid only for this fantasy world and the fantasy birds.

Conclusions: In conclusion, a reexamination is needed of the actual existence of an aposematic aspect to the coloration patterns in Heliconiinae, Ithomiinae, *D. plexippus* (L.), and other Lepidoptera, and indeed the whole hypothetically co-evolved physiological system and avian detection of chemicals hypothetically serving a defensive function in this butterfly-predator system. The system outlined by so many authors in the mimicry literature, based on quite limited experimental tests of artificially constrained predators, well-orchestrated experimental designs, and quite free-ranging expectations of neat "just so" explanations of natural systems, may be deeply flawed. An alternative argument that tasting the prey is beneficial for both the prey and the predator (the prey safely escapes and the predator is not exposed to a lethal dose by eating the toxic prey) is too much an altruistic belief to be valid.

The progress of science proceeds on rails made of hypotheses and theories that are disputed, lu-

bricated by tolerance for consideration of different points of view. This critical essay is contributed in the belief that it is time to re-examine the long-held beliefs and widespread passive acceptance of certain aspects of mimicry theory in a key animal group, and perhaps thereby progress to a new and better level of understanding.

Appendix: Some confusion is created by the way the ED50 of CDGs (one emetic dose) is calculated. An ED50 dose is defined as the weight of dried monarch material, ground to a fine homogenous powder, placed in a gelatin capsule and forced down the throat into the empty gizzard of blue jays (*Cyanocitta cristata* CR.), which causes the bird to vomit 50% of the time (BROWER et al.,1968). The cuticle-bound CDGs are poorly digested (CLEMENT, 1977) and the cardenolides mixture stored in the monarch's cuticle is so tightly bound that much of it is defecated before the bird can absorb an emetic dose (BROWER et al. 1988). Evidently, the amount of CDGs absorbed from a swallowed whole body, before the cuticle is expelled, is only a small fraction of the amount that would be absorbed if the body entered the empty gizzard in the form of a fine grounded powder that markedly facilitates the digestive process. Thus, the amount of CDGs calculated from the powder form of monarch material does not correspond to that same amount in the body swallowed by the bird intact. The actual intact-body supplied amount of CDGs must be significantly higher - the bird must consume far more monarchs than the theoretically calculated number. Because the process of absorption of the CDGs depends on many variables, there is no meaningful constant concentration of the alkaloids in jays that induces vomiting. One emetic unit can be a constant amount only when measuring the amount of CDGs in a plant or comparing the amount of CDGs contained in different plants, and the plant material is processed in the same way as that of the body parts of the Heliconiinae species.

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